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Identification of starch granules using a two-step identification method

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ABSTRACT

Starch analysis has proven to be a powerful method applicable to recover microbotanical remains of starchy foods in archaeological contexts, and morphometric analysis is the most commonly used methodological approach for identifying starch granules. However, it is sometimes not easy to achieve a high level of accuracy in identification, if several coexisting taxa in an assemblage exhibit similar starch morphology. The current study attempts to use both traditional morphometric observation and also a computer-based discriminant analysis to create a multivariate model, in order to separate Job's tears (*Coix lacryma-jobi*) from foxtail millet (*Setaria italica* ssp. *italica*) and broomcorn millet (*Panicum miliaceum*) that show considerable overlapping in starch morphology and size. The two-step identification method generated in this study shows a greater power of discrimination for identifying these three taxa with high success rates. The model was then used for identification in ancient assemblages with satisfactory efficiency and accuracy. This method will be most useful for application to ancient starch assemblages recovered from sites where dry-land farming was a significant part of the subsistence strategy, such as in East Asian Neolithic and Bronze Ages.

1. Introduction

Starch analysis has proven to be a powerful method applicable to recover microbotanical remains of starchy foods in archaeological contexts, and morphometric analysis is the most commonly used methodological approach for identifying starch granules (Reichert, 1913, 1919; Torrence and Barton, 2006). However, it is sometimes not easy to achieve a high level of accuracy in identification, if several coexisting taxa in an assemblage exhibit similar starch morphology. Researchers have encountered such problems when starch remains may derive from several genera belonging to the grasses of the Panicoideae subfamily. The most frequently occurring Panicoideae plants with economic importance in the Chinese archaeological record include Job's tears (*Coix lacryma-jobi* in the Andropogoneae tribe), foxtail millet (*Setaria italica* ssp. *italica*), and broomcorn millet (*Panicum miliaceum*) (both in the Paniceae tribe), whose starch granules show considerable overlapping in morphology and size. Some attempts have been made to separate these taxa in modern contexts (Ge et al., 2010, Yang et al., 2012b), but identification in ancient assemblages is often ambiguous.

Foxtail millet and broomcorn millet are known to have been the first cereals cultivated in north China about 10,000 years ago (Yang et al., 2012a; Zhao, 2014), while Job's tears is a much less understood cereal, and its domestication process is currently unclear. Job's tears, often cultivated, is widely distributed in East, Southeast and South Asian subtropical and temperate regions today, used as food, medicine, and decoration (Chen and Phillips, 2006). Seeds of Job's tears, which were preserved mostly in water-logged or extremely dry conditions, have been discovered only at a small number of sites in China. The earliest remains come from Hemudu in Zhejiang (5000–4000 BC) (Zhejiang Provincial Institute, 2003:217), Chengtoushan in Hunan (4400–4200 BC) (Liu and Gu, 2007), Baodun in Sichuan (2700–2000 BC) (Guedes et al., 2013), and Sampula in Xinjiang (ca. 50 BC) (Jiang et al., 2008). Starch granules identifiable as Job's tears, however, have been recovered more frequently from pottery and grinding stones in sites dating to the sixth millennium BC in both southern and northern China (Liu et al., 2013b; Yang and Jiang, 2010; Zhang et al., 2011b). Job's tears may have not survived well as macroremains in the archaeological record for reasons that are currently uncertain; it nevertheless appears to have been exploited, in addition to millets, by early Neolithic populations.

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Therefore, starch analysis can be an important alternative method by which to recover verifiable samples of this plant. Nevertheless, due to substantial overlapping in starch granule size and morphology of these three Panicoideae plants, the identification and recovery of Job's tears is far from certain when using traditional methods. It is necessary therefore to develop new methods for separating Job's tears from millets in starch remains, in order to better understand the processes of plant usage and cereal domestication in China.

Multivariate techniques, such as discriminant analysis, have been employed to set up a system of classification for starch granules, in order to make a preliminary study of a large number of starchy plants present in Papua New Guinea. The results show that dichotomous variables play the largest role in classification, followed by starch size and then shape (Torrence et al., 2004). Discriminant analysis has also been used in phytolith analysis to distinguish domesticated from wild rice (Zhao, 1998; Zhao et al., 1998) and millet (Zhang et al., 2011a) in China. This method shows great potential for development of a statistical modeling strategy usable to identify ancient starch granules. The current study attempts to use both traditional morphometric observation and also discriminant analysis (using IBM SPSS Statistics 21) to create a multivariate model, in order to separate Job's tears from millets in ancient assemblages with reasonable efficiency and accuracy.

2. Materials and methods

We selected samples of domesticated Job's tears, wild Job's tears, foxtail millet, and broomcorn millet from our modern reference collection, which contains more than 900 samples collected from China and other regions in East Asia. Each selected taxon included multiple samples from different regions (Table 1).

Job's tears show a range of variation in fruit type, shape and size. There are two species of Job's tears with several botanically recognized variants in China as recorded in *Flora of China*: *Coix lacryma-jobi* Linnaeus (*yiyi* in Chinese) with four variants, and *Coix aquatic* (*shuisheng yiyi* in Chinese). The cultivated *C. lacryma-jobi* var. *lacryma-jobi* exhibits a bony utricle and is grown for decorative purposes (e.g., as beads for making necklaces), while *C. l.* var. *ma-yuen* has a thinner utricle and is produced for food and medicine (Chen and Phillips, 2006). It has been suggested that wild forms of *Coix* have harder and thicker utricles, and the cultivated thin-utricle races of *Coix* originated through gene mutation from the wild forms as a result of conscious selection by humans for easy husking (Arora, 1977). In the current study, we selected both wild and domesticated Job's tears for analysis; the domesticated ones included both bony-utricle and thin-utricle types (Table 1).

Discriminant analysis, or linear discriminant function analysis, is a statistical method that builds a predictive model for group membership. The model is composed of a discriminant function based on linear combinations of predictor variables, which provide the best discrimination between groups. The purpose of discriminant analysis is to maximally separate the groups and to determine the most parsimonious way to separate groups. The original data, for which group membership (plant taxon in this case) is known, is divided into two groups, a training set and a test set. The model is generated from the training set, and the discriminant power of the variables is evaluated by Wilks' Lambda index (0 denotes perfect discriminatory power and 1 means no discriminatory power). The model is then applied to the test set for validation. Finally the model is applied to the archaeological data, using measurements from the predictor variables for discriminant grouping.

In the current study, the training set, including 12 samples (three from each group), was used to generate the discriminant model. The test set was composed of four samples (one from each group) for evaluating the effectiveness of the model (Table 1).

3. Analysis and results

3.1. Selection of predictor variables

Starch granules from Job's tears and millets are generally small in size; facetted, round, or oval in shape; and exhibit linear-, V-, Y-shaped or radiated fissures (Fig. 1). We selected four predictor variables that are potentially diagnostic for identification. These variables include: (1) size of the granule (SG), meaning the maximum length of a starch granule measured through the hilum; (2) eccentricity of the hilum (EH), meaning the ratio of the linear distance between the hilum and the edge of the granule on both sides (long arm/short arm); (3) presence or absence of a Z-shaped arm (ZA) on the extinction cross, meaning that under the polarized filter, a clearly defined angular and double-curved shape is present on one (or more) of the four arms; and (4) presence or absence of curved arm(s) (CA) on the extinction cross, which differs from the Z-shaped arm in that only one curve is present on an arm (Fig. 1; see also Supplementary fig. 1 for more detailed illustration). These variables on each granule were measured and recorded in Excel, and then transferred to SPSS for statistical analysis.

Table 1
Modern samples used in discriminant analysis.

	Lab ref#	Domesticity	Origin	Size range (mean) in μm	Maximum eccentricity
Training set					
Job's tears	1250-14	Dom. thin utricle	Hebei	7.77–20.05 (13)	2.36
Job's tears	1252-14	Dom. utricle unkn.	Heilongjiang	5.72–17.67 (12.56)	2.44
Job's tears	1244-13	Dom. bony utricle	Guizhou	6.09–**29.2** (15.27)	2.36
Job's tears	1243-13	Wild, bony utricle	Hunan	7.11–19.68 (12.25)	2
Job's tears	1260-14	Wild, bony utricle	Yunnan	6.42–20.31 (13.04)	**2.53**
Job's tears	1261-14	Wild, bony utricle	Yunnan	7.28–**20.89** (14.49)	1.89
Foxtail millet	662-09	Domestic	Shanxi	5.29–16.53 (10.71)	**1.44**
Foxtail millet	664-09	Domestic	Shaanxi	7.04–**21.17** (8.87)	1.44
Foxtail millet	665-09	Domestic	Henan	7.01–20.34 (10.77)	1.44
Broomcorn millet	183-08	Domestic	Liaoning	4.49–10.52 (7.37)	1.44
Broomcorn millet	977-09	Domestic	Shaanxi	3.16–11.73 (6.48)	1.38
Broomcorn millet	666-09	Domestic	Shanxi	3.03–12.34 (8.35)	**1.47**
Test set					
Job's tears	1254-14	Dom. thin utricle	Taiwan	7.37–23.84 (15.11)	**2.69**
Job's tears	1263-14	Wild, bony utricle	Yunnan	4.54–18.65 (12.37)	1.68
Foxtail millet	822-09	Domestic	China	4.84–14.49 (9.73)	1.38
Broomcorn millet	661-09	Domestic	Shaanxi	4.19–**12.8** (7.59)	1.47

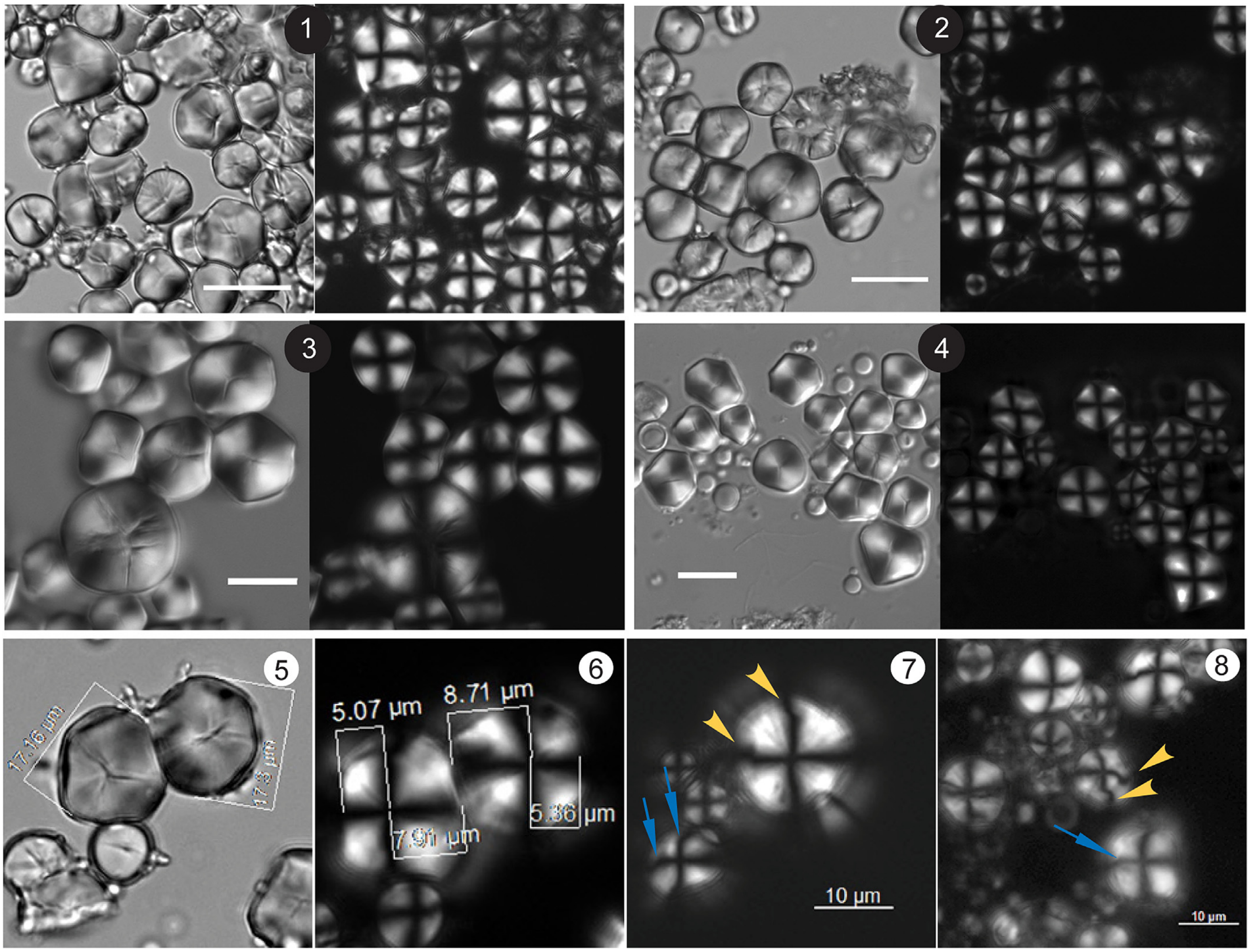

Fig. 1. Starch granules from modern reference and morphometric attributes used in the analysis. 1: Job's tears (domesticated, Hebei); 2: Job's tears (Yunnan, wild); 3: foxtail millet; 4: broomcorn millet; 5: size measurements; 6: eccentricity measurements; 7 & 8: Z-shaped arm (pointed with arrow without stem) and curved arm (pointed with stemmed arrow) on the extinction cross (5–8 from Job's tears; scale 1,2: 20 μm; 3,4:10 μm).

A preliminary examination of these predictor variables has suggested that some features are more diagnostic than others for identification.

1. SG: In order to cover as many regional variations as possible in starch size, we measured SG in nearly all the available samples of the four taxa in our collection, at least 100 granules from each sample. These included four samples from domesticated Job's tears, four from wild Job's tears, eight from foxtail millet, and ten from broomcorn millet. We are aware of that modern Job's tears and millets from different regions often exhibit different starch sizes, and our reference samples are collected from limited regions. However, the starch maximum lengths from the same species in reference samples collected from other regions, including nine foxtail millet samples and nine broomcorn millet samples (Yang et al., 2012a), are similar to the data used in the current project (see Supplementary table 1), suggesting that our data are reasonably representative. Ancient starch residues have often been recovered from grinding stones, and starch granules tend to enlarge in size after grinding (Liu et al., 2013a). Therefore, we included samples that had been measured after grinding, so that our model is applicable to archaeological starch assemblages. It is notable that after grinding some starch granules become broken or distorted in form, a situation which would make the size measurements difficult and unreliable. Such badly damaged starch granules might also not survive in archaeological contexts. To overcome this problem, we avoided taking measurements from those badly damaged starch granules which were deformed, or had lost part or all of the extinction cross. After taking these considerations, the greatest size from each group was selected as the cut-off score: 29.2 μm for domesticated Job's tears, 20.89 μm for wild Job's tear, 21.17 μm for foxtail millet, and 12.8 μm for broomcorn millet (Table 1; shown in bold). Accordingly, if a starch granule from one of the three taxa under study is larger than 21.17 μm in size, it can be identified as domesticated Job's tears.
2. EH: Four samples from each type (wild and domesticated) of Job's tears and five from each type of millet, with more than 50 granules from each sample, were measured. The results show that both wild and domesticated Job's tears exhibit higher eccentricity ratio (2.69 for domesticated and 2.53 for wild) than millets (1.47 for broomcorn and 1.44 for foxtail) (Table 1; shown in bold). Therefore, if a starch granule from one of the three taxa shows an eccentricity ratio greater than 1.47, it can be identified as Job's tears, either wild or domesticated.

Table 2
Presence of Z-shaped arm (ZA) on the extinction cross of starch granule in Job's tears.

Sample	Total granules	Granules with ZA	Percentage of ZA
Dom. Job's tears (Guizhou)	57	6	10.53%
Dom. Job's tears (Hebei)	66	10	15.15%
Dom. Job's tears (Heilongjiang)	70	16	22.86%
Dom. Job's tears total	**193**	**32**	**16.58%**
Wild Job's tears (Hunan)	55	1	1.82%
Wild Job's tear (Yunnan 1260)	53	1	1.89%
Wild Job's tear (Yunnan 1261)	77	4	5.19%
Wild Job's tear total	**185**	**6**	**3.24%**
Foxtail millet (Shanxi)	55	0	1.82%
Foxtail millet (Shaanxi)	48	1	2.08%
Foxtail millet (Inner Mongolia)	54	0	1.85%
Foxtail millet (Henan)	50	0	0
Foxtail millet total	**207**	**1**	**0.48%**
Broomcorn millet (Shanxi)	56	1	1.82%
Broomcorn millet (Shaanxi 1)	55	0	0
Broomcorn millet (Shaanxi 2)	50	0	0
Broomcorn millet (Liaoning)	53	1	1.89%
Broomcorn millet total	**214**	**2**	**0.93%**

3. ZA: This feature is present most frequently in domesticated Job's tears (16.58% of 195 granules from three samples), less so in wild Job's tears (3.24% of 185 granules from three samples), rare in broomcorn millet (.93% of 214 in four samples), and nearly absent in foxtail millet (.48% of 207 in four samples) (Table 2). Therefore, if ZA is present on a starch granule from one of the three taxa under study, it can be tentatively identified as Job's tears, either wild or domesticated. This feature should be used together with SG and EH for identification in a starch assemblage. The presence of ZA on larger granules is more likely to belong to Job's tears than that on very small granules (particularly in the range of broomcorn millet). However, since a very small percentage of starch granules in millets exhibits ZA, the presence of ZA alone in an assemblage cannot be used for identification of Job's tears if SG and EH do not meet the cut-off scores for Job's tears. Further analysis is required in this situation (see below).
4. CA: Domesticated Job's tears exhibits the highest frequency of curved arms, followed by wild Job's tears, broomcorn millet and foxtail millet (Table 3). This feature was used as a variable for discriminant analysis.

Table 3
Frequencies of curve on arm of the extinction cross from different taxa.

Samples	Total granules measured	Granules with curve(s)	Percentage
Dom. Job's tears (Guizhou)	57	18	31.58
Dom. Job's tears (Hebei)	66	24	36.36
Dom. Job's tears (Heilongjiang)	70	57	81.43
Dom. Job's tears average	**64**	**33**	**51.30**
Wild Job's tears (Hunan)	55	18	32.73
Wild Job's tears (Yunnan 1260)	53	13	24.53
Wild Job's tears (Yunnan 1261)	52	28	53.85
Wild Job's tear average	**53**	**20**	**36.88**
Foxtail millet (Shanxi)	55	10	18.18
Foxtail millet (Shaanxi)	48	6	12.5
Foxtail millet (Inner Mongolia)	54	4	7.41
Foxtail millet (Henan)	50	5	10
Foxtail millet average	**51.75**	**6.25**	**12.08**
Broomcorn millet (Shaanxi)	56	6	10.71
Broomcorn millet (Shaanxi 1)	55	19	34.55
Broomcorn millet (Shaanxi 2)	50	14	28
Broomcorn millet (Liaoning)	53	10	18.87
Broomcorn millet average	**53.5**	**12.25**	**22.9**

Table 4
Classification matrix from wild and domestic Job's tears.

4.1. Classification Results[a]

Group		Predicted group membership		Total
		Wild Job's tears	Dom. Job's tears	
Count	Wild Job's tears	**66**	24	90
	Dom. Job's tears	109	**95**	204
%	Wild Job's tears	**73.3**	26.7	100.0
	Dom. Job's tears	53.4	**46.6**	100.0

4.2. Tests of equality of group means

	Wilks' Lambda	F	df1	df2	Sig.
Eccentricity	.967	9.679	1	286	.002
Curve	1.000	.000	1	286	1.000
Size	.998	.669	1	286	.414

[a] 54.8% of original grouped cases correctly classified.

3.2. First-step analysis: preliminary identification

Based on the above analysis, variables 1–3 (SG, EH, ZA) were found to be the most decisive diagnostic criteria for separating Job's tears from millets. A preliminary identification for Job's tears and millets based on morphometric observation can be made on the basis of the following criteria:

- A starch granule is identifiable as Job's tears, probably domesticated, if it is larger than 21.17 μm in size.
- A starch granule is identifiable as Job's tears (wild or domesticated) if it has an eccentricity ratio greater than 1.47,
- A starch granule is identifiable as Job's tears (wild or domesticated) if it has a Z-shaped arm on the extinction cross, particularly on larger granules (>12.8 μm for broomcorn millet).

Granules that do not meet any of these criteria are then subjected to secondary examination by discriminant analysis. These are granules with a size ≤21.17 μm, an eccentricity ratio ≤1.47, and absence of Z-shaped arm on the extinction cross. If the general size range of an assemblage is small, and a Z-shaped arm only occurs on very small granules, such a granule should be classified as curved (CA) and analyzed in the statistical model.

3.3. Second-step analysis

3.3.1. Building discriminant model

Three variables were used for building the discriminant model: SG, EA, and CA. Thirty granules from each sample were measured.

Table 5
Results of discriminant analysis based on training samples.

5.1. Classification results[a]

Species		Predicted group membership			Total
		Broomcorn millet	Foxtail millet	Dom. Job's tears	
Count	Broomcorn millet	**62**	28	0	90
	Foxtail millet	25	**58**	7	90
	Dom. Job's tears	1	19	**70**	90
%	Broomcorn millet	**68.9**	31.1	.0	100.0
	Foxtail millet	27.8	**64.4**	7.8	100.0
	Dom. Job's tears	1.1	21.1	**77.8**	100.0

5.2. Tests of equality of group means

	Wilks' Lambda	F	df1	df2	Sig.
Eccentricity	.763	40.061	2	258	.000
Size	.415	182.077	2	258	.000
Curve	.979	2.712	2	258	.068

[a] 70.4% of original grouped cases correctly classified.

Table 6
Results of blind test.

Original species		Blind-test group membership			Total
		Broomcorn millet	Foxtail millet	Job's tears	
Count	Broomcorn millet	**34**	8	0	42
	Foxtail millet	8	**29**	0	37
	Job's tears (w&d)	0	16	**75**	91
%	Broomcorn millet	**81**	19	.0	100.0
	Foxtail millet	21.6	**78.4**	.0	100.0
	Job's tears (w&d)	.0	17.6	**82.4**	100.0

*80.6% of original grouped cases correctly classified.

In order to understand whether starch granules of wild and domesticated Job's tears can be distinguished from each other, we first examined these two groups (three samples from each group) with discriminant analysis. The results show that the success rate for wild Job's tears is 73.3%, but for domesticated Job's tears it is only 46.6% (Table 4.1; shown in bold). The statistical analyses included in the table titled, Tests of Equality of Group Means, measure each independent variable's potential before the model is created. Each test displays the results of a one-way ANOVA for a specific independent variable using the grouping variable as the factor. If the significance value is greater than .10, the variable does not contribute to the model. According to the results shown in Table 4.2, the only significant variable in our discriminant model was eccentricity. Wilks' Lambda is another measure of a variable's potential. Smaller values indicate that the variable more effectively discriminates between groups. The table suggests that eccentricity is the best variable. Therefore, only one independent variable (eccentricity) is available for building the model. However, one variable cannot indicate all the granular differences between wild and domesticated Job's tears. There are probably some other variables that, together with eccentricity, can be used to recognize the morphological differences of those starch granules more accurately. In the current study, these two groups cannot be separated effectively. More efforts are needed to improve the model in the future. As a result, we removed wild Job's tears from further analytical processes.

3.3.2. Training set

The training set of data consists of three species: domesticated Job's tears, foxtail millet, and broomcorn millet. We analyzed three samples for each species, and 30 granules for each sample. The results show that success rates are relatively high, with 68.9% for broomcorn millet, 64.4% for foxtail millet, 77.8% for Job's tears, and 70.4% on average (Table 5.1); shown in bold.

Among the three variables (size SG, eccentricity EH, and curve CA) used for analysis, size (SG) has the highest discriminant power, indicated by a relatively low value in Wilks' Lambda (.415) and a high F value (182.077), followed by eccentricity (EH). Curve (VA) is the weakest variable, with Wilks' Lambda near 1 and a rather low F value. Nevertheless, all three variables show values of significance lower than .1, meaning that they all contribute to the model (Table 5.2).

3.3.3. Validation test

To validate the discriminant model we conducted a blind test. The test set was composed of four groups (wild and domesticated Job's tears and two millets), one sample from each group, and 30

Table 7
Application of the two-step identification method to ancient starch from Zhaigen and Shihushan.

Site	Granule size	Size >21.17	Z	Short arm	Long arm	Eccentricity	Curve	1st-Step ID	Discriminant analysis	Final ID
Zhaigen	20.2	0	1					Job's tears		Job's tears
	16.8	0	0	6.12	10.65	1.74		Job's tears		Job's tears
	18	0	0	9.32	9.83	1.05	1		Job's tears	Job's tears
	17.28	0	0	6.26	7.99	1.28	1		Job's tears	Job's tears
	13.62	0	0	5.1	6.39	1.25	0		Job's tears	Job's tears
	16.94	0	1					Job's tears		Job's tears
	19.48	0	0	6.77	9.65	1.43	0		Job's tears	Job's tears
	19.59	0	1					Job's tears		Job's tears
	15.77	0	0	4.71	6.71	1.42	1		Job's tears	Job's tears
	24.43	1	0					Job's tears		Job's tears
	16.82	0	0	6.36	8.26	1.30	1		Job's tears	Job's tears
	16.73	0	0	8.21	8.68	1.06	0		Job's tears	Job's tears
	19.43	0	0	7	8.04	1.15	1		Job's tears	Job's tears
	13.95	0	0	5.91	7.49	1.27	0		Job's tears	Job's tears
	12.28	0	0	5.62	6.82	1.21	1		Job's tears	Job's tears
	19.4	0	0	7.69	9.32	1.21	1		Job's tears	Job's tears
	20.21	0	0	7.42	9.86	1.33	0		Job's tears	Job's tears
	20.67	0	0	7.26	13.05	1.80		Job's tears		Job's tears
	26.4	1	1					Job's tears		Job's tears
	21.46	1	1					Job's tears		Job's tears
	12.72	0	0	4.26	5.99	1.41	0		Job's tears	Job's tears
	20.09	0	0	4.61	9.66	2.10	1	Job's tears		Job's tears
	25.69	1	0					Job's tears		Job's tears
	22.22	1	0					Job's tears		Job's tears
	17.27	0	0	6.63	9.15	1.38	1		Job's tears	Job's tears
	17.86	0	0	6.99	9.26	1.32	0		Job's tears	Job's tears
Shihushan	14.44	0	0	6.94	6.47	1.07	0		Job's tears	Job's tears
	12.47	0	0	6.98	6.19	1.13	0		Foxtail millet	Foxtail millet
	9.24	0	0	4.8	4.35	1.1	1		Broomcorn millet	Broomcorn millet
	15.67	0	0	7.57	7.26	1	0		Job's tears	Job's tears
	19.51	0	0	10.1	7.73	1.31	1		Job's tears	Job's tears
	18.49	0	0	9.62	8.52	1.13	0		Job's tears	Job's tears
	7.69	0	0	3	3	1	0		Broomcorn millet	Broomcorn millet
	7.95	0	0	3.16	2.85	1.11	0		Broomcorn millet	Broomcorn millet
	13.76	0	1					Job's tears		Job's tears

granules from each sample. The starch images were first examined in a preliminary identification, directed at singling out the granules immediately identifiable as Job's tears, and the examiner was not informed about the group identities of the images. Then the remaining data were entered into the training-set file in SPSS as an unclassified group for discriminant analysis. The results from the two-step process (preliminary identification followed by discriminant analysis) show high success rates, with 82.4% correction for Job's tears (including both wild and domesticated), 78.4% for foxtail millet, 81% for broomcorn millet, and 80.6% on average (Table 6; shown in bold). These results suggest that Job's tears can be clearly separated from broomcorn millet; but Job's tears and foxtail millet, as well as foxtail and broomcorn millets, cannot be completely separated. In general, a great majority of each taxon (around 80%) can be discriminated successfully. The two-step identification procedure proves to be efficient and effective.

3.4. Examining ancient samples

To further test the effectiveness of the two-step discriminant model described above, we applied it to two ancient starch assemblages, which were previously identified as Job's tears and millet.

The first assemblage is derived from the Zhaigen site (ca. 6000–5000 BC) of the Peiligang culture in Henan. A grinding slab from the site revealed a large number of starch grains, among which 26 were identified as Job's tears, based on size (12.72–26.4 μm) and general morphology. Since Job's tears and millet overlap in regard to shape of starch granules, the original identification in the report was unable to rule out the possible presence of millet in the assemblage (Liu et al., 2013b). When the criteria in the first-step analysis were applied to this assemblage, 11 granules were immediately identifiable as Job's tears. These are five granules larger than 21.17 μm in size (including two with ZA), three additional granules (size range 16.94–20.2 μm) exhibiting ZA, and three exceeding the cut-off score for EH (1.47). When the discriminant model was applied to the remaining 15 granules, all of them were identified as Job's tears (Table 7). This result confirmed and raised the confidence level of the original identification.

The second assemblage is from the Shihushan II site (ca. 5000–4500 BC) of the Yangshao culture in Liangcheng, Inner Mongolia. A total of 363 starch granules were recovered from seven grinding stones. Nine granules were identified as millet, based on size (7.69–19.51 μm) and general morphology (Liu et al., 2014). When the two-step discriminant model was applied to this assemblage, five granules were classified as Job's tears (including one with ZA [13.76 μm] which was singled out in the first-step analysis), one as foxtail millet, and three as broomcorn millet (Table 7). This result suggests that some of ancient starch assemblages from north China, which were previously identified as millets, may actually include Job's tears and can be reanalyzed with the method developed in this study to improve the accuracy of identification.

The application of the model in these two cases demonstrates its great power for producing more refined identifications by which to distinguish Job's tears from millets in archaeological assemblages.

4. Discussion and conclusions

The two-step starch identification method discussed in this study combines traditional morphological-size observation and a computer-based discriminant analysis. The preliminary identification is effective for singling out the most readily diagnosed granules, while the secondary discriminant analysis helps achieve a higher level of accuracy to classify the remaining, less directly diagnosable granules. Together the method shows a greater power of discrimination for identifying Job's tears, foxtail millet, and broomcorn millet with high success rates. This method will be most useful for application to ancient starch assemblages recovered from sites where dry-land farming was a significant part of the subsistence strategy, such as in East Asia of the Neolithic and Bronze Ages.

In the current analysis, size appears to be the most useful variable, and the dichotomous features, which reflect very specific values (e.g., presence or absence of Z-shape arm on the distinction cross) are also effective in the first-step identification. This observation is similar to the conclusion derived from the previous study on Papua New Guinea materials (Torrence et al., 2004).

Separating domesticated Job's tears from its wild counterpart remains problematic. Additional variables may be required to achieve a better outcome in the future. It will be particularly useful to study the process of Job's tear domestication, about which we know little at present.

The current study indicates that a multivariable approach, combining both traditional morphometric observation and statistical analysis, is an effective method in starch analysis. This method can be employed to target those confusing and ambiguous taxa for better identification in ancient starch analysis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2014.09.008.

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